Eurobiofilms 2015 - Educational Workshop

Growth and imaging techniques for laboratory and clinical biofilm research

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Workshop Objectives

• Appropriate selection of biofilm growth system
  • Identify experimental objectives
  • Hydrodynamic parameters relevant to biofilms

• Staining Biofilms for microscopic examination
  • Viability
  • FISH

• Data capture
  • Image rendering (Imaris)
  • Image Analysis (COMSTAT)
9:00 – 9:15 Welcome and introduction to the faculty.

9:15 – 9:45 Biofilm laboratory growth systems - an overview
Paul Stoodley, (The Ohio State University, Department of Microbial Infection and Immunity, Columbus, OH, USA)

9:45 – 10:15 Working with clinical specimens and FISH
Kasper Nørskov Kragh (University of Copenhagen, Department of Systems Biology, Copenhagen, Denmark)

10:15 – 10:30 Refreshment break (Coffee, tea, water, snacks provided in room)

10:30 – 11:00 Rendering and analyzing confocal biofilm images (the differences between image rendering and analysis)
Claus Sternberg, (Technical University of Denmark, Department of Systems Biology Lyngby, Denmark)

11:00 – 11:30 Panel discussion - Audience specific interest and systems - questions about the theory.
11:30 – 12:30 Split into 3 groups – each group at a separate workstation.

Group 1 – Flow cells
Group 2 – FISH and viability staining
Group 3 – Imaris and COMSTAT

12:30 – 1:30 Lunch

1:30 – 2:30
Group 1 – Imaris and COMSTAT
Group 2 – Flow cells
Group 3 – FISH and viability staining

2:30 – 3:30
Group 1 – FISH and viability staining
Group 2 – Imaris and COMSTAT
Group 3 – Flow cells

3:30 – 3:45 Refreshment break and wrap up Q/A discussions (Coffee, tea, water, snacks provided in room)

4:00 Conference Opening ceremony AULA AUDITORIUM
Biofilm laboratory growth systems - an overview

Paul Stoodley, (The Ohio State University, Department of Microbial Infection and Immunity, Columbus, OH, USA)
Why use flow cells? They are not high throughput and can be difficult to manage.

Biofilm formation is a developmental process
Avoid use of air water solid interface which destroys biofilm during a rinse step.

Open system more closely mimics real systems.

Can control shear stress.

Can follow perturbations and pulse chase in real time.
Biofilms – Wide Range of Shear Stresses

Marine Biofouling
- Hulls
- Structures

Host tissue
- Infective endocarditis
- CF lung

Nosocomial
- Catheters
- Dental water lines

Public Health
- Drinking water
Biofilms in Industry

Problems
Product contamination
Public health / spoilage
Membrane plugging
Corrosion
Energy losses in pipelines
SRB oil souring
Emphasis on Imaging or Sampling?

Imaging: two basic systems “once through” or “recirculating”

Sampling: CDC reactor, Robbins device, annular reactor (rototorque)
Flow Cell / Reactor Design

Hydrodynamics – Shear and Nutrient Loading Rate

Magnification range – scales of observation
Reactor wall thickness may limit high definition high power microscopy

Surface of interest – transparent or opaque?

Sampling required?
Flow Cells for Biofilm Research

Microscope and image analysis *in situ*

- Media Carboy
- Bubble Trap
- Pump
- FC-81 Flowcell
- Waste Carboy
- Flow break
“Once-through” System

Key
- Flow meter
- Adjustable clamp
- Silicone tubing
- Flow break
- Sampling port
- Inoculation port

Video camera
Microscope objective

Flow cell

Peristaltic pump
Flow meter
Nutrients
Vane head pump
Recirculation loop
Mixing chamber

Waste
Air pump and filter
Recirculating System

Key:
- Flow meter
- Adjustable clamp
- Silicone tubing
- Flow break
- Sampling port
- Inoculation port

Diagram:
- Video camera
- Microscope
- Objective
- Flow meter
- Adjustable clamp
- Silicone tubing
- Flow break
- Sampling port
- Inoculation port
- Flow cell
- Peristaltic pump
- Recirculation loop
- Nutrients
- Recirculation loop
- Vane head pump
- Mixing chamber
- Air pump and filter
- Waste
Flow cell operating requirements

High resolution microscopy – requires flat surface and a transparent window

Channel width limited by objective working distance, limits flow rate

Opaque coupon – i.e. steel
Flow cell operating requirements

High resolution microscopy – requires flat surface and a transparent window

Channel wall width limited by objective working distance, limits rigidity
Microscopy

Bright field (phase / interference contrast)

Epi-fluorescent

Reflected (interference contrast)

Confocal (3D fluorescent / transmitted / reflected)
What can we measure?

Microscopically
- Surface area coverage
- Thickness
- Length dimensions of biofilm structures
- Count objects
- Brightness / Florescence Intensity
- Rates of change
- Molecular tools - GFP expression
- Material properties

External monitoring
- Pressure drop
- Effluent cell count
- Biofilm cell count (end point)
Recirculating System

The residence time ($\theta$) can be controlled by the nutrient flow rate ($Q_n$) according to $\theta = V/Q_n$, where $V =$ the volume of the mixing chamber plus the recirculation loop.

The flow rate in the flow cells can be adjusted independently of the nutrient flow rate so that much higher flow rates can be achieved in the flow cells without using impractical volumes of media.

$Q_n$ can be adjusted so no planktonically dividing population by making the dilution rate ($D$) greater than the growth rate ($\mu$)

$$D = Q_n/V \quad \mu = \ln(2)/Dt$$
Hydrodynamic characterization

Shear force caused by fluid flow

No slip condition (no water flow at the wall)
Hydrodynamic Parameters for Closed Channel

Flow rate \((Q)\) ml/min or \(m^3/s\)

Flow velocity \((u_{ave})\) = \(Q/CSA\) (m/s)

Channel cross sectional area, \(CSA = hw (m^2)\)

Wetted perimeter, \(WP = 2w + 2h\) (m)

Reynolds number = \(u_{ave} \rho L_{ch}/\eta\)

\(L_{ch} = 4D_h = 4CSA/WP\)

\(\eta\) absolute viscosity
\(\rho\) density
\(\nu\) kinematic viscosity = \(\eta/\rho\)
Hydrodynamic Parameters for Closed Channel II

Reynolds number

<500 laminar (low shear)
500 – 1500 transition
> 1500 turbulent (high shear)

\( \eta \) absolute viscosity
\( \rho \) density
\( \nu \) kinematic viscosity = \( \eta / \rho \)
Pressure drop as fluid moves down a tube due to friction

\[ f = \frac{\Delta P \times D_h}{2l_f \rho_w u_{(ave)}^2} \]
Biofilm Flow Cell Hydrodynamics

Pressure drop ports
Friction factor and shear stress

\[ f = \frac{16}{Re} \]  
Laminar flow

\[ f = 0.0791/Re^{0.25} \]  
Blasius equation for turbulent flow

Shear force caused by fluid flow
Shear stress

\[ \tau_w = \frac{f \rho_w u_{(ave)}^2}{2} \]  \hspace{1cm} \text{Turbulent flow (empirical)}

\[ \tau_w = \frac{4\eta u_{(max)}}{D_h} \]  \hspace{1cm} \text{Laminar flow (analytical solution)}

Square or circular tube \( u_{\text{max}} = 2 \ u_{\text{ave}} \)
Flat plate \( u_{\text{max}} = 3/2 \ u_{\text{ave}} \)
\( D_h = L_{ch} = \text{hydraulic diameter} \)
Adhesion Assays

316 Stainless Steel

Fluropolymer

P. aeruginosa PAO1
Growth Assay
Mechanical Assays

Deformation $n = f$ shear
Antibiofilm Agents

Anti-Biofilm Performance of Three Natural Products against Initial Bacterial Attachment

Maria Salta ¹, Julian A. Wharton ¹, Simon P. Dennington ¹, Paul Stoodley ¹ and Keith R. Stokes ¹,²
Adhesion / Detachment Shear Assays
Shear induced detachment

Flow velocity (m/s)

Cluster surface concentration (cm$^{-2}$)

Time (s)

$U_{(\text{grow})}$

$U_{(\text{detac})}$
Expression of GFP was only observed in the biofilms after flow was turned off.

P. aeruginosa pMH509

*lasB::GFP*

5-day old biofilm,
Q=1ml/min
Thank You to BioSurface Technologies for Supplying Flow Cell Materials

Questions?